Enhanced antibacterial and anti-adhesive activities of silver–PTFE nanocomposite coating for urinary catheters

Zhang, Shuai; Wang, Liyun; Liang, Xinjin; Vorstius, Jan; Keatch, Robert; Corner, George

Published in:
ACS Biomaterials Science and Engineering

DOI:
10.1021/acsbiomaterials.9b00071

Publication date:
2019

ACS Biomaterials SCIENCE & ENGINEERING

Article

Cite This: ACS Biomater. Sci. Eng. 2019, 5, 2804−2814

pubs.acs.org/journal/abseba

Enhanced Antibacterial and Antiadhesive Activities of Silver-PTFE Nanocomposite Coating for Urinary Catheters

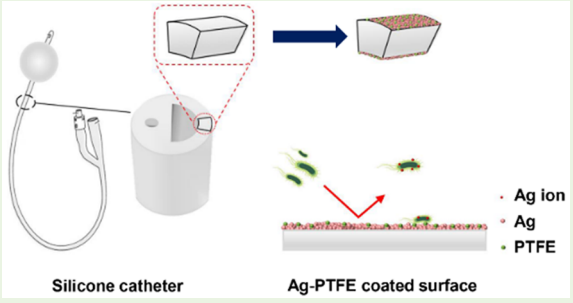
Shuai Zhang,[†] Liyun Wang,[†] Xinjin Liang,[‡] Jan Vorstius,[†] Robert Keatch,[†] George Corner,[†] Ghulam Nabi,[§] Fordyce Davidson,[†] Geoffrey Michael Gadd,[‡] and Qi Zhao*[,†]

[†]School of Science and Engineering, University of Dundee, Dundee DD1 4HN, United Kingdom

[‡]Geomicrobiology Group, School of Life Sciences, University of Dundee, Dundee DD1 5EH, United Kingdom

[§]Academic Section of Urology, School of Medicine, Ninewells Hospital, Dundee, DD1 9SY, United Kingdom

ABSTRACT: Catheter-associated urinary tract infection (CAUTI) presents a significant health problem worldwide and is associated with increased morbidity and mortality. Herein, a silver-polytetrafluoroethylene (Ag-PTFE) nanocomposite coating for catheters was developed via a facile wet chemistry method. Benefiting from the synergistic effect of Ag and PTFE, the as-prepared Ag-PTFE-coated catheter exhibited enhanced antibacterial and antiadhesive activities against two CAUTI-associated strains: *E. coli* WT F1693 and *S. aureus* F1557. Compared to the uncoated commercial silicone catheters and the Ag-coated catheters, the Ag-PTFE-coated catheters were able to reduce bacterial adhesion by up to 60.3% and 55.2%, respectively. The Ag-PTFE-coated catheters also exhibited strong antibiofilm activity, reducing biofilm coverage by up to 97.4% compared with the commercial silicone catheters. In an in vitro bladder model, the Ag-PTFE-coated catheter displayed excellent anti-infection efficacy against bacteriuria, extending the lifetime of silicone catheters from a mean of 6 days to over 40 days. The Ag-PTFE coating also showed good biocompatibility with fibroblast cells in culture, making it a prospective strategy to overcome current challenges in CAUTI.



KEYWORDS: *urinary catheter, infections, coatings, antibacterial, silver, polytetrafluoroethylene*

INTRODUCTION

Catheter-associated urinary tract infection (CAUTI) is one of the most common healthcare-acquired infections (HAI) accounting for approximately one-third of all device-related infections and 40% of infections hospital-wide.[1−3] The significant incidence of such infection stems from the fact that the urinary catheter provides access for bacteria from the outside environment directly to the urinary tract while impairing the normal defense mechanisms of the bladder making the catheter susceptible to bacterial adhesion and colonization.[4,5] Further, biofilms readily develop on inner and outer surfaces of the catheters giving a survival strategy for bacteria.[6,7] These biofilms are complex differentiated communities comprising multiple associations of cells and extracellular polymeric substances (EPS) that function as both barriers to antibiotics and reservoirs of pathogenic bacteria.[8,9] As a result, the bacterial biofilms can persist as a continuing nidus for infection. In practice, 10−50% of patients undergoing short-term catheterization (up to 7 days) develop a urinary tract infection (UTI) and nearly all patients during long-term catheterization (over 28 days) become infected.[10]

Current commercialized strategies for preventing CAUTI, apart from improving sterile techniques, involve coating with antibacterial materials such as antibiotics, silver, or antiadhesive materials like hydrogels and polytetrafluoroethylene (PTFE).[11−13] However, recent clinical studies have shown none of these coated catheters are able to reduce symptomatic CAUTI significantly compared with standard catheters.[14,15] Furthermore, the use of antibiotic-based coatings may result in an increased antibiotic resistance.[16] Unlike antibiotics, silver (Ag) is a nonspecific bactericide that can act against a broad spectrum of bacterial species even at low concentrations, but previous studies have shown that the Ag-coated catheters can only delay the early onset catheter-related infections, as evinced by the increase in bacteriuria after 1 week of catheterization.[17] Observations on the infected catheter demonstrate that a "foundation layer" composed of both dead and live bacteria can rapidly form on the silver-coated surface, which allows bacteria to colonise and grow, protected from contact with the underlying silver.[18,19] Lubricating coatings such hydrogels improve patient's comfort by remarkably decreasing the friction between the catheter and the urethral tissue, but they also facilitate the migration of urinary tract pathogens over the catheter surface.[20] PTFE, due to its inherent nonstick properties, was hoped to be an ideal material for catheter coatings. However, there is no clear

Received: January 16, 2019
Accepted: May 2, 2019
Published: May 2, 2019

DOI: 10.1021/acsbiomaterials.9b00071
ACS Biomater. Sci. Eng. 2019, 5, 2804−2814

evidence that the PTFE-coated catheters are superior to standard catheters as the rippled surface of PTFE coatings could predispose the catheter to infection by providing sites for bacterial attachment.[13]

To overcome the existing problems in marketed catheters, we developed a silver-PTFE (Ag-PTFE) nanocomposite coating for urinary catheters by incorporating PTFE nanoparticles into the Ag matrix via a wet-chemistry method. The antibacterial efficiencies of the Ag-PTFE-coated catheter were systematically investigated against two CAUTI-related strains *Escherichia coli* and *Staphylococcus aureus* in terms of bacterial adhesion, bactericidal efficacy, and long-term stability. The antibiofilm ability of the coated catheters was also compared to two types of marketed catheters: PTFE-coated and all-silicone. To verify the efficacy against bacteriuria and bacterial migration, the coating was evaluated in a clinically relevant CAUTI bladder model and to test biocompatibility, the cytotoxicity of the coated catheters was evaluated with L929 mouse fibroblast cells.

EXPERIMENTAL SECTION

Preparation of Ag-PTFE-Coated Urinary Catheters. Commercially available all-silicone Foley catheters (Mediplus Ltd., Bucks, U.K.) with a diameter of 5.3 mm (16 Fr) were cut into 2 cm long segments and sequentially rinsed with ultrasonication in absolute ethanol (Sigma-Aldrich, Gillingham, U.K.) and ultrapure water (Milli-Q, Darmstadt, Germany). The Ag and Ag-PTFE-coated catheters were then prepared according to the previous methods.[21,22] For the Ag-PTFE coating, in order to obtain a uniform dispersion of PTFE particles in the plating bath, 5 mg/L of PTFE emulsion (60.0 wt % with PTFE particle size in the range 0.05−0.5 μm, Sigma-Aldrich, Gillingham, U.K.) and 0.2 g/L of FC-4 cationic surfactant (Guangang Technology, Shanghai, China) were added and the mixture was thoroughly mixed by sonication for 10 min at room temperature.[23] The obtained samples were rinsed with 0.1 M HNO_3, Milli-Q water, and ethanol in sequence and finally air-dried at room temperature. The full-length of all-silicone Foley catheters was also coated by the same process.

Surface Characterization. A scanning electron microscope (field emission-scanning electron microscope (FE-SEM), JEOL JSM-7400F, Tokyo, Japan) was employed to study the surface morphology of coatings using an accelerating voltage of 10 kV. Prior to observation, the samples were vacuum-dried and sputter-coated with gold/palladium (Au/Pd) with a 208HR high-resolution Sputter Coater (Cressington Scientific Instruments Inc., Watford, U.K.). The particle size distribution was calculated from random SEM images for triplicated specimens using ImageJ. An atomic force microscope (AFM) with a Nanoscope III Scanning Probe Microscope controller (Dimension 3000, Santa Barbara, CA, U.S.A.) was used to examine the surface roughness of the coatings. All AFM measurements were operated in a tapping mode using a diamond-like carbon (DLC)-coated silicon cantilever (spring constant, 320 kHz). Roughness values were obtained from images of scan size 5 μm × 5 μm and analyzed using NanoScope Analysis 1.10 software. For the surface composition analysis, energy-dispersive X-ray spectrometry (EDX, QX200, Bruker Ltd., Billerica, U.S.A.) was used at an accelerating voltage of 15 kV. The distributions of Ag and PTFE were monitored by EDX elemental mapping across the entire surface of the coatings. Contact angle measurement was conducted by using a sessile drop method with a Dataphysics OCA-20 contact angle analyzer (DataPhysics Instruments GmbH, Filderstadt, Germany) the surface energies of coatings were calculated using the van Oss approach.[24] All measurements were performed at 25 °C and the contact angles on at least six replicates of each sample were collected to get the mean values and standard deviation.

Initial Bacterial Adhesion. The antiadhesion efficacy of the Ag-PTFE coating against Gram-negative *E. coli* WT F1693 and Gram-positive *S. aureus* F1557 was assessed using fluorescence microscopy. For adhesion tests, cultures of *E. coli* WT F1693 and *S. aureus* F1557 were grown to the midexponential phase (~2 × 10^8 CFU/mL) and diluted with phosphate-buffered saline (PBS) to a concentration of ~5 × 10^7 CFU/mL because cells harvested in this growing phase have shown the best adhesion to a solid surface. Each of the uncoated (control) and coated (sample) catheter segments (n = 6) were immersed vertically in 5 mL of the prepared bacterial suspension and incubated at 37 °C and 30 rpm for 24 h. At the end of the incubation, the catheter segments were rinsed gently with sterile tris buffered saline (TBS) to remove any loosely bound bacteria, followed by staining with a LIVE/DEAD Baclight bacterial viability kit L13152 (Fisher Scientific, Loughborough, U.K.) in the dark for 15 min. A fluorescence microscope (OLYMPUS BX 41, Tokyo, Japan) was used to observe the adhered bacterial cells and their viability and the adhered cell numbers were quantified using Image Pro Plus software (Media Cybernetics, Rockville, U.S.A.).

To study long-term stability, some Ag-PTFE-coated catheter segments (n = 6) were dried thoroughly and exposed in air under daylight at room temperature and their antiadhesion efficacy at day 14 was then tested as described above.

Ag^+ Release and Antibacterial Assays. To examine the behavior of Ag^+ release from the Ag and Ag-PTFE-coated catheters, samples (n = 3) were immersed in 5 mL of 1× PBS (pH 7.4) at 37 °C and the total Ag^+ concentrations at 1, 2, 3, 5, 7, 10, and 14 days were quantified using atomic absorption spectrometry (PerkinElmer, AAnalyst 400, U.S.A.). Samples were filtered through 0.45 μm pore size membrane syringe filter (Sartorius Stedim Biotech, Germany) and stored at 4 °C before analysis. Calibration standards of 0, 1, 3, 5, and 10 μg/L of Agwere prepared from a 1000 mg/L standard from TraceCERT (Sigma-Aldrich, U.K.). Triplicate readings were analyzed for each sample and control samples of known silver concentration were analyzed in parallel, showing acceptable recoveries of added amounts (95−100%). Any difference in means between treatments was determined by one-way analysis of variance (ANOVA) to a 0.05 significance level.

Earlier studies suggest that Gram-negative and Gram-positive bacteria may exhibit different sensitivities toward Ag^+ and a low concentration of Ag^+ is insufficient to kill bacteria.[25] To investigate if a continuous Ag^+ release from the coatings results in an attenuated antibacterial efficacy, we studied their antibacterial efficacy against the planktonic bacteria in suspensions over a period of 14 days. In this assay, each sample after the above test was reincubated with 5 mL of freshly prepared bacterial suspension for 24 h, and the viable bacteria in suspension were quantified by standard dilution and plate-counting. The antibacterial efficacies (i.e., the number of killed bacteria) from day 0 to day 14 were then determined by calculating the difference between the mean numbers of viable bacteria in the suspensions of control and samples (n = 6).

Biofilm Adhesion. The antibiofilm efficacy of the Ag-PTFE-coated catheters (n = 3) was compared with BARD PTFE-coated Foley catheters and Mediplus all-silicone Foley catheters by incubating in 5 mL of *E. coli* or *S. aureus* suspensions (TSB solution, ~2 × 10^8 CFU/mL) at 37 °C and 30 rpm for 48 h. The bacterial suspension was refreshed at 24 h. After 48 h, each sample was washed gently three times with TBS and fixed with 2.5% glutaraldehyde at 4 °C for 12 h and dehydrated sequentially in 30%, 50%, 70%, 90% and absolute ethanol for 10 min in each. Prior to SEM observation, all the samples were dried and sputter coated with gold/platinum.

Bacterial Migration Assays with Full-Length Coated Catheters. To investigate the ability of Ag-PTFE-coated catheters to resist bacterial migration along the catheter surface, an in vitro bladder model was used.[26] According to Nicolle et al.,[27] a single catheterized urine specimen with one bacterial species isolated in a quantitative count ≥10^2 CFU/mL identifies bacteriuria in women or men. Therefore, in this study a bacterial suspension (~10^2 CFU/mL of *E. coli* WT F1693) was prepared in 5% dextrose solution as the source of infection. The presterilized catheter was fixed through a funnel which represented the urethra meatus and exposed to bacterial contamination originating from the source of infection dripping over

DOI: 10.1021/acsbiomaterials.9b00071
ACS Biomater. Sci. Eng. 2019, 5, 2804−2814

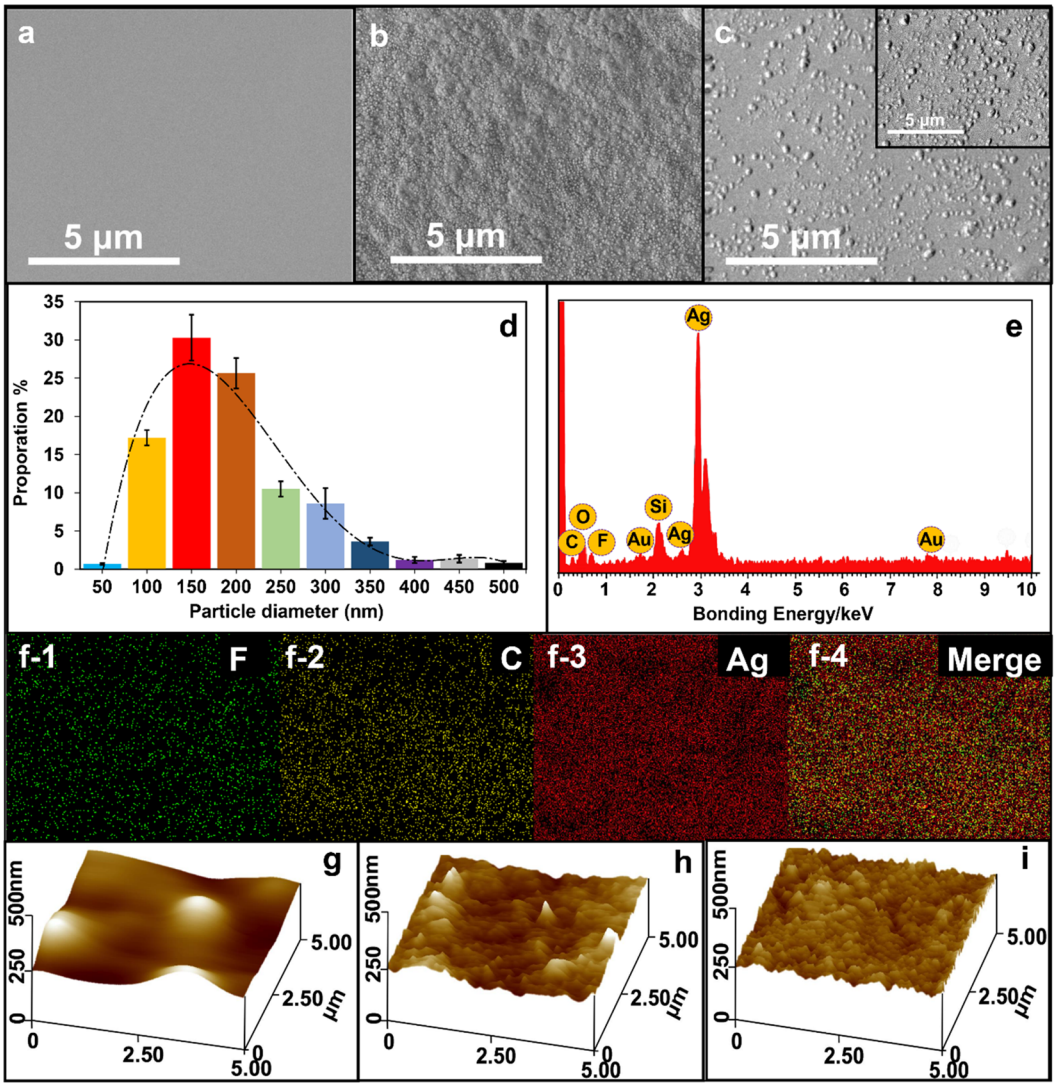


Figure 1. Morphology and microstructure characterization of coatings. SEM images of (a) silicone catheter; (b) Ag-coated catheter; (c) Ag-PTFE-coated catheter; (d) size distribution of the PTFE particles; (e) semiquantitative results of EDX; (f) EDX mapping of the SEM image C; AFM images of (g) silicone catheter; (h) Ag-coated catheter; (i) Ag-PTFE-coated catheter. Typical images are shown from several examinations (bars represent standard deviation of the mean).

the external surface of the catheter at a fixed rate of 0.2 mL·min^{-1}. In order to mimic the urine flow around the catheter, sterile artificial urine was dripped via a peristaltic pump at a steady rate of 36 mL·h^{-1} into the "bladder" and drained through the catheter lumen and the "urethra meatus". The occurrence of bacteriuria (detectability limit, $\geq 10^3$ CFU/mL) can be determined from the density of bacteria in urine by using a plated count technique. The average time taken for the occurrence of bacteriuria for the coated and uncoated catheters was recorded. Moreover, the biomass formed along the whole length of the catheters was quantified by crystal violet staining.[28]

Cytotoxicity Assay. Cytotoxicity tests were performed according to the ISO 10993-5:2009(E), using mouse fibroblast cells L929 obtained from European Collection of Authenticated Cell Cultures (U.K.), commonly used for cytotoxicity evaluation of biomaterials.[29] All the samples were carefully sterilized in 70% ethanol before test. The L929 cell lines were cultured in Eagle's minimum essential medium (MEM) supplemented with 10% fetal bovine serum in addition with 100 mg/mL penicillin and 100 mg/mL streptomycin for 24 h at 37 °C, 5% CO_2. After achieving the confluence, 500 μL of the cell suspension with ~10^5 cells/mL were seeded in each well of a 48-well plate. Then, the cells were incubated in a humidified atmosphere (>90% humidity) with 5% CO_2 at 37 °C overnight. The samples (n = 6) were carefully placed into the 48-well plate and incubated for 24, 48, and 72 h, respectively. Wells containing only the cells were used as control. The cytotoxicity was measured using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay method. All the medium was removed and 50 μL of MTT was added was added to each well. After 4 h of incubation in the dark at 37 °C, 500 μL of isopropanol was added to each well to dissolve the formazan. The absorbance was measured at $OD_{570\ nm}$ and relative cell viability was measured by comparison with the control.

Cell Morphology. For the cells in culture medium, the change in cell morphology after exposure to the samples was examined using light microscopy (Leica DMIL, Solms, Germany) and the images were taken after 24, 48, and 72 h of incubation. For the cells attached on the samples, after culturing for 72 h, the samples were taken out and rinsed three times with 1× PBS, fixed with 2.5% glutaraldehyde at 4 °C overnight, and dehydrated in graded ethanol for 10 min each. The dehydrated samples were then critical-point dried, gold/platinum coated, and examined by SEM.

Statistical Analysis. Statistical analysis was performed using SPSS software (version 19.0) and data were represented as the means ± standard deviation. Group comparison was conducted using a one-way ANOVA combined with a Student−Newman−Keuls (SNK) post hoc test to determine the level of significance. $p < 0.05$ was considered significant and $p < 0.01$ was considered highly significant.

DOI: 10.1021/acsbiomaterials.9b00071
ACS Biomater. Sci. Eng. 2019, 5, 2804−2814

Table 1. Contact Angle and Surface Energy Components of Different Catheters

samples	contact angle, θ (deg)			surface free energy (mJ/m^2)			
	θ^W	θ^D	θ^E	γ^{LW}	γ^+	γ^-	γ^{TOT}
silicone	108.2 ± 0.4	77.1 ± 0.1	97.8 ± 1.0	19.00	0.54	0.04	19.29
PTFE	112.3 ± 0.8	78.3 ± 0.9	92.9 ± 1.3	18.37	0.03	0.11	18.49
Ag	70.9 ± 1.3	52.8 ± 1.5	47.7 ± 0.7	32.7	0.39	13.34	37.28
Ag-PTFE	90.3 ± 1.8	74.2 ± 0.9	83.1 ± 1.4	20.56	0.09	7.15	22.18

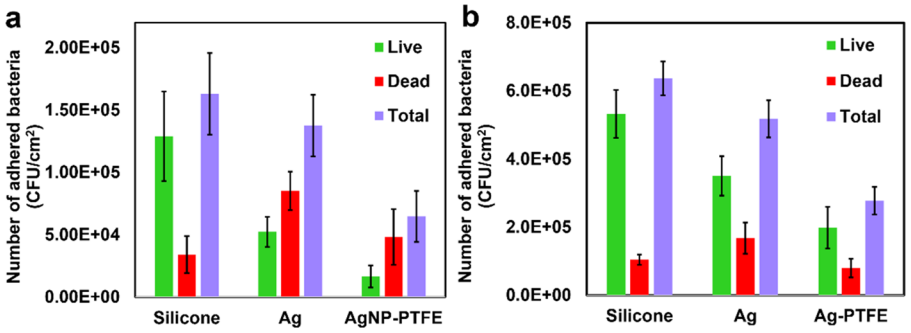


Figure 2. Quantitative counts of adhered live and dead (a) *E. coli* (b) *S. aureus* on different surfaces after 24 h incubation (bars represent standard deviation of the mean).

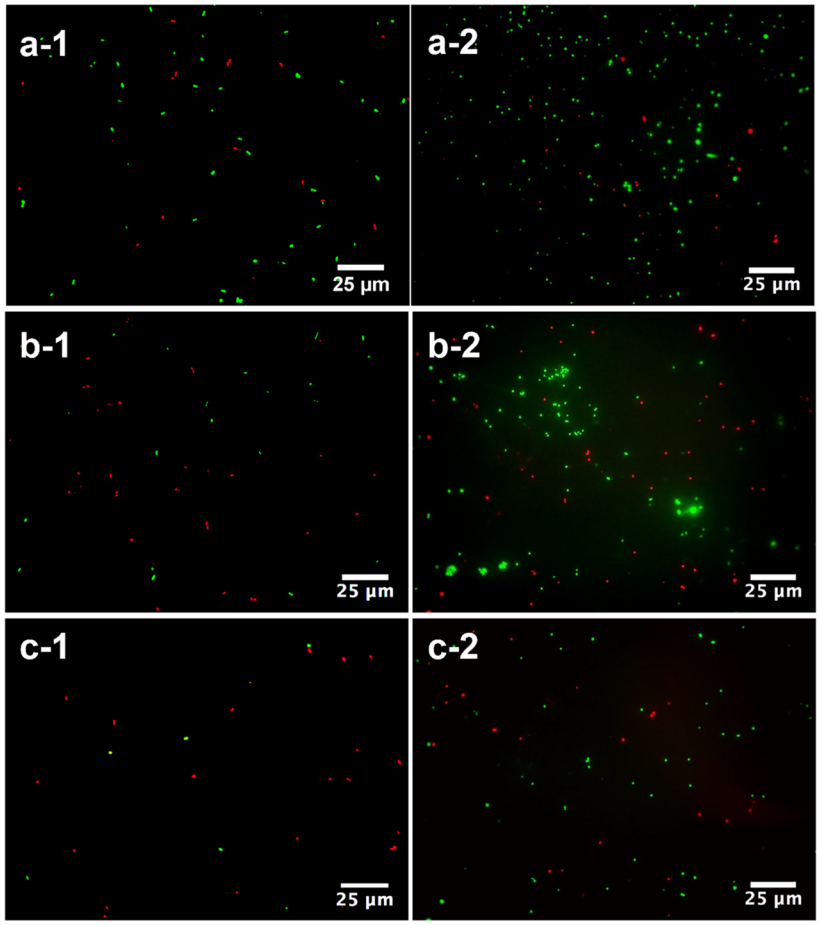


Figure 3. Live/dead fluorescent images of adhered (left) *E. coli* and (right) *S. aureus* on (a) silicone surface, (b) Ag, and (c) Ag-PTFE-coated surfaces after 24 h incubation.

RESULTS AND DISCUSSION

Surface Characterization. Surface morphology of the coatings was characterized using SEM analysis. The uncoated silicone surface (Figure 1a) clearly presented a smoother morphology compared to the coated ones. For the Ag-coated catheter surface, Ag particles with a size range of 50−200 nm were uniformly distributed over the whole catheter surface (Figure 1b). Further incorporation of PTFE particles into the

DOI: 10.1021/acsbiomaterials.9b00071
ACS Biomater. Sci. Eng. 2019, 5, 2804−2814

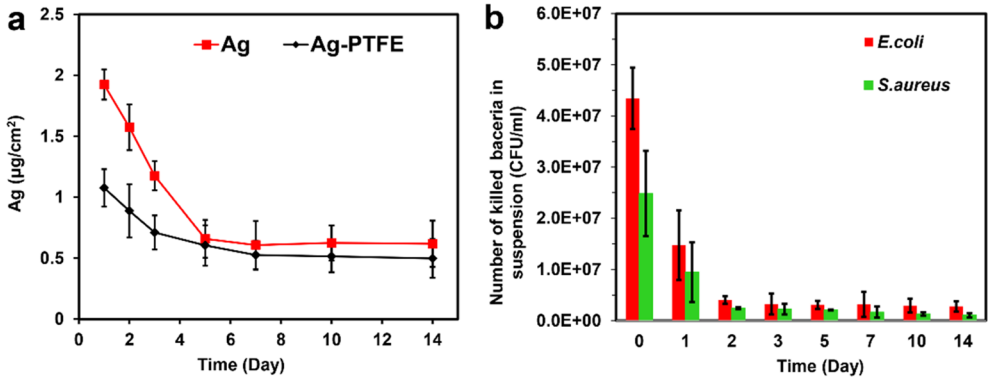


Figure 4. (a) Silver ion released ($\mu g/cm^2$) from the Ag coating and Ag-PTFE coating versus time in 5 mL 1× PBS. (b) Quantitative counts of killed planktonic *E. coli* and *S. aureus* cells.

Ag matrix yielded a rougher surface (Figure 1c). The PTFE particles with an average diameter of 150 nm (Figure 1d) were uniformly distributed and buried in the Ag matrix. Figure 1e shows a typical surface composition of the Ag-PTFE coating. The major surface constituents included Ag, C, O, F, Si, and Au. The Au derived from the gold-sputtering before SEM observation and the Si was from the silicone substrate as the coating thickness was less than 1 μm. Figure 1f-1, f-2, f-3, and f-4 show the distributions of Ag, C, O, and F in the coatings obtained by EDX elemental mapping, which further verifies the uniform distribution of PTFE particles throughout the coating. AFM results confirmed that bare silicone surface possessed the smoothest morphology. After Ag and Ag-PTFE coating processes, the surface roughness increased to 29.4 ± 5.7 nm (Figure 1h) and 69.3 ± 7.3 nm (Figure 1i) compared to the uncoated silicone surface (17.8 ± 2.3 nm) (Figure 1g).

The surface wettability affects biofunctions such as bacteria/cell adhesion and spreading.[30] In this study, this was evaluated by the static sessile drop method. As seen in Table 1, the water contact angle of the Ag-coated surface significantly decreased to 70.9 ± 1.3° compared to the uncoated surface (108.2 ± 0.4°). After incorporating hydrophobic PTFE particles into the Ag matrix, a sensible increase in hydrophobicity was obtained (90.3 ± 1.8°), which is in agreement with data reported in our previous studies.[21,31]

Initial Bacterial Adhesion. The early adherence of the two most common uropathogens (Gram-negative *E. coli* and Gram-positive *S. aureus*) to the uncoated, Ag-coated and Ag-PTFE-coated catheters was evaluated by the fluorescence microscopy. As seen in Figures 2 and 3, the Ag-PTFE-coated catheters exhibited the lowest bacterial adherence for both *E. coli* and *S. aureus*. Compared to the uncoated silicone catheters and the Ag coated catheters, the Ag-PTFE-coated catheters reduced adhesion of *E. coli* by 60.3% ($p < 0.05$) and 55.2% ($p < 0.05$), respectively, and reduced adhesion of *S. aureus* by 56.5% ($p < 0.05$) and 49.1% ($p < 0.05$), respectively. Moreover, the Ag-PTFE-coated catheters also exhibited antibacterial efficacy against the adhered bacteria. For *E. coli*, Figure 2a,c demonstrated that most of the adhered cells on uncoated silicone surface (control) were green (alive) and only a few (20.9%) were red (dead). In comparison, 74.5% of the adhered cells on the Ag-PTFE-coated catheters were dead. As to *S. aureus*, the ratio of dead bacteria on the coated catheters (25.6%) was also significantly higher than that on the control (16.4%) ($p < 0.05$). This could be attributed to the release of silver ions (Ag^+) that killed some of the adhered bacteria. Interestingly, the Ag-coated catheters showed similar antibacterial effects to the Ag-PTFE-coated catheters, but their antiadhesive efficiency was significantly lower when compared to the Ag-PTFE-coated catheters ($p < 0.05$). The enhanced antiadhesive property of the Ag-PTFE coating can be ascribed to the nonstick property of PTFE.

Ag^+ Release and Stability of the Ag-PTFE Coating. Figure 4a displays the Ag^+ release from the Ag and Ag-PTFE-coated catheters coatings as a function of immersion time up to 14 days. In general, relatively large amounts of Ag^+ were released in the initial 3 days. Afterward, the amounts of released Ag^+ gradually decreased and became constant after 5 days. In comparison, the total amount of Ag^+ released from the Ag-coated catheters in the first 3 days was significantly higher than that from the Ag-PTFE-coated catheters ($p < 0.05$). As time elapsed, the release rates became close and reached a relative steady value of ~0.55 $\mu g/cm^2$. In order to identify the antibacterial effects of the released Ag^+ against the planktonic bacteria, a plate-counting technique was used and the results were shown in Figure 4b. For *E. coli*, ~4×10^7 CFU/mL bacteria in suspension was killed by the newly prepared Ag-PTFE-coated catheter (0 day immersion in 1x PBS). Afterward, a gradual decrease in antibacterial efficacy was observed for the Ag-PTFE-coated catheter as a function of immersion time. After 2 days in immersion, the released Ag^+ was still able to kill ~3×10^6 CFU/mL *E. coli*, and the antibacterial efficacy was still maintained even up to 14 days in immersion. Similar phenomenon was also observed in *S. aureus*.

The stability of the Ag-PTFE-coated catheters was tested by examining their antiadhesion and antibacterial efficacies after exposure to air for 14 days. The experimental results demonstrated that no significant decrease in antiadhesive and antibacterial efficacies was observed with both *E. coli* and *S. aureus* (data not show). Early studies have pointed out that immobilization of Ag particles onto a substrate could prevent/retard their oxidation, which could be responsible for the good stability and long-term antimicrobial activity of the Ag-PTFE coatings.[32,33] These results indicated that the Ag-PTFE-coated catheters could not only retard bacterial adhesion but also effectively kill planktonic bacteria, which might satisfy long-term anti-infective needs.

Antibiofilm Efficacy. The antibiofilm efficacy of the Ag-PTFE-coated catheter was examined and compared with two types of commercial catheters (BARD PTFE-coated Foley catheters and Mediplus all-silicone Foley catheters) through a

DOI: 10.1021/acsbiomaterials.9b00071
ACS Biomater. Sci. Eng. 2019, 5, 2804−2814

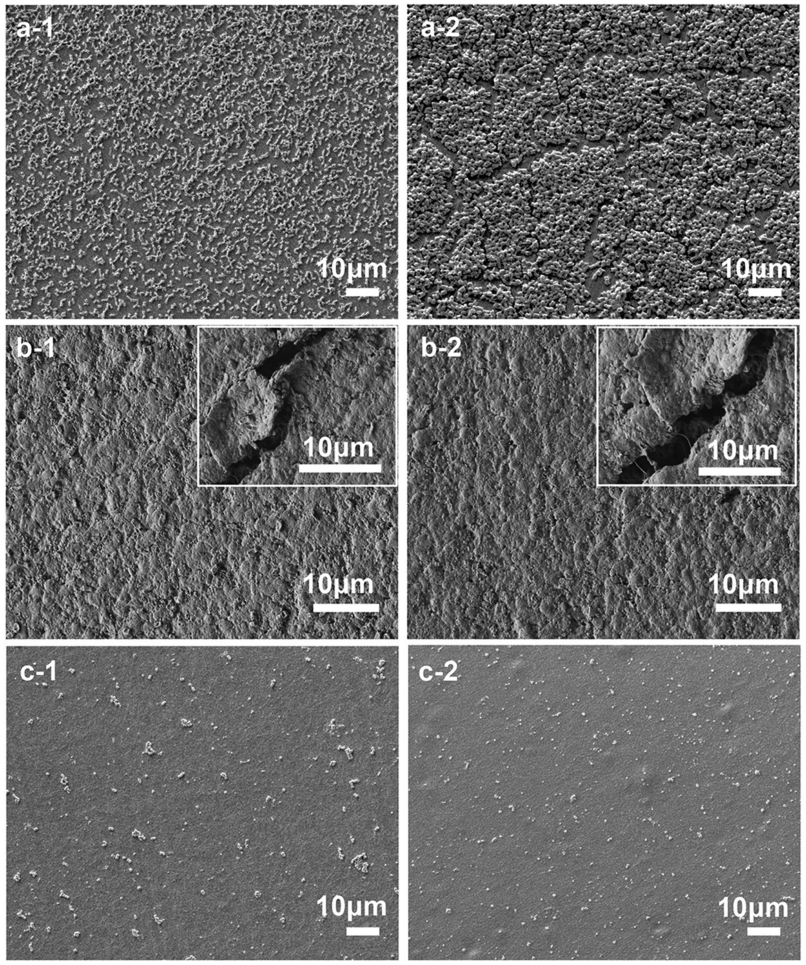


Figure 5. Biofilm formation on different surfaces after 48 h. (a-1) *E. coli* on silicone catheter; (a-2) *S. aureus* on silicone catheter; (b-1) *E. coli* on PTFE coated catheter; (b-2) *S. aureus* on PTFE coated catheter; (c-1) *E. coli* on Ag-PTFE-coated catheter; (c-2) *S. aureus* on Ag-PTFE-coated catheter surface. Typical images are shown from several examinations.

prolonged culturing of 48 h, by means of SEM. As illustrated in Figure 5a–c, for both *E. coli* and *S. aureus*, the uncoated silicone catheters showed the highest levels of biofilm formation. The PTFE-coated catheters exhibited a slight improvement in resisting biofilm formation while a vast number of bacteria were still found (Figure 5b1 and 5b2). Notably, a significant number of bacteria were trapped in the rippled surface structure of the PTFE-coated catheters and this would predispose the catheter to infection by providing ideal sites for bacterial adhesion.[13] The Ag-PTFE-coated catheters (Figure 5c1 and 5c2) were mostly free from bacterial colonization and no trace of mature biofilm was found. Compared to the uncoated silicone catheters and the PTFE coated catheters, the Ag-PTFE-coated catheters reduced the biofilm coverage of *E. coli* by 95.2% ($p < 0.05$) and 92.4% ($p < 0.05$), respectively, and reduced the biofilm coverage of *S. aureus* by 97.4% ($p < 0.05$) and 96.2% ($p < 0.05$), respectively.

Efficacy of the Ag-PTFE-Coated Catheters against Bacterial Migration and Growth. The efficacy of Ag-PTFE-coated full-length catheter against bacterial migration was assayed with *E. coli* via an in vitro bladder model (Figure 6a). This model has proven to be very effective in simulating the infection process, and it allows for the easy detection of bacteriuria. As seen in Figure 6b, upon challenge with ~10^2 CFU/mL of *E. coli*, it took a significantly longer time to cause bacteriuria following bacterial contamination of the external surface of the Ag-PTFE-coated catheter (a mean of 40 days) versus uncoated silicone catheter (a mean of 6 days). To further investigate the effectiveness of Ag-PTFE coating against bacterial growth along the catheter surfaces, the catheters following bacteriuria were retrieved from the system and sectioned longitudinally into 10 pieces with 2 cm each, and the total amount of biomass accumulated onto the catheter surface was determined by Gram's crystal violet staining assay. Apparently, as seen in Figure 6c, the uncoated silicone surface showed significantly higher level of total biomass accumulation than the Ag-PTFE-coated surface. Interestingly, for the uncoated silicone surface, the biomass accumulation showed a "Normal distribution" that over 54% of the biomass was located in the middle sections. In comparison, the amount of biomass on the Ag-PTFE-coated catheter surface gradually increased from the infection site to the balloon. The results were also demonstrated by fluorescence microscopy (Figure 6d). These findings suggest that all-silicone Foley catheters coated with Ag-PTFE can effectively impede the migration and growth of a urinary pathogen (*E. coli*) along the catheter surface. As clinical results have suggested that the silicone catheter can be used alone for up to 12 weeks,[34] it is therefore expected that the Ag-PTFE-coated catheter will provide

DOI: 10.1021/acsbiomaterials.9b00071
ACS Biomater. Sci. Eng. 2019, 5, 2804–2814

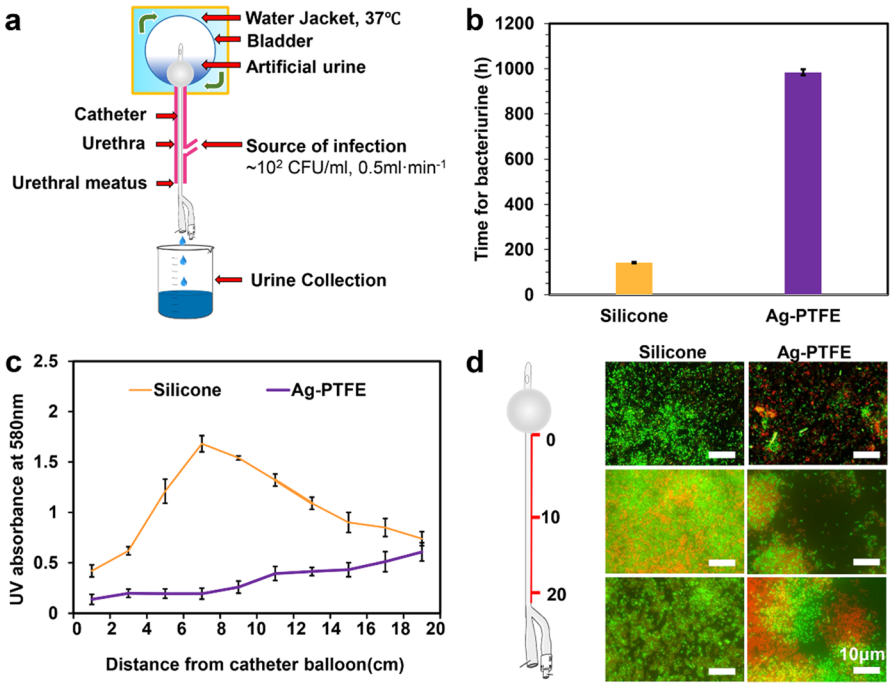


Figure 6. (a) Illustrative diagrams of the in vitro bladder model; (b) comparisons of time taken for the occurrence of bacteriuria of uncoated versus coated catheters; (c) UV absorbance (OD_{580}) data for different sections of uncoated and Ag-PTFE-coated catheters after the occurrence of bacteriuria, determined by crystal violet staining assay; (d) representative fluorescence microscopy images of bacteria/biofilm adhered along the external surfaces of uncoated and Ag-PTFE-coated catheters. All values shown are means of at least three measurements and typical images are shown from several examinations (bars represent standard deviation of the mean).

improved protection against CAUTI and will persist for a longer period during catheterization.

Cytotoxicity and Cell Attachment. The biocompatibility of a coating material is an essential aspect with regard to its potential clinical translation and cell culture studies are usually the first step to investigate the potential toxicity of these coatings.[35] In this study, the cytotoxicity of the samples to L929 mouse fibroblast cells was examined using direct contact method as fibroblasts might contact the catheter surface while indwelling. As shown in Figure 7a, following 24 h of coincubation, the viability of the cells appeared higher in the silicone catheters (control). For the Ag and Ag-PTFE-coated catheters, both cell viabilities were over 82% and no significant difference was noticed. As incubation time increased, wells containing Ag-coated catheters showed a slight decrease in cell viability, reaching 77.9% and 75.1% after 48 and 72 h of incubation, respectively. In comparison, the cell viabilities of the Ag-PTFE-coated catheters were higher and relatively stable (about 82%) throughout the culturing period, indicating the improved biocompatibility of the Ag-PTFE coating. Collectively these results confirmed the nontoxic properties of the Ag and Ag-PTFE coatings in accordance with ISO norms, as all the cell viabilities were over 70%.

Figure 7b compares the morphologies of cells after 24, 48, and 72 h of direct contact with different samples. As the culture time increased, the number of cells gradually increased in all groups and no significant difference in cellular morphology was observed, which further verified that the Ag and Ag-PTFE coatings have no cytotoxicity and support cell proliferation. The SEM images in Figure 7c shows the morphologies of cells cultured on different surfaces for 72 h. The fibroblast cells distributed homogeneously on all the samples and adhered tightly to the surfaces with dense filopodia, which could be beneficial to cell proliferation. It was observed that the cells developed a spindle-like morphology with single long cell arms on the surface of the uncoated silicone catheter, whereas the cells cultured on the Ag and Ag-PTFE-coated catheters tended to spread with a healthy flattened shape. Generally, hydrophobic surface is considered to be negative to mammalian cells.[36] After coating with Ag and Ag-PTFE, the hydrophilicity of the silicone substrate was significantly improved, which might favor cell adhesion and spreading.

Discussion. The present study aimed to prepare an anti-infection coating for urinary catheters incorporating the strengths of two strategies: to kill bacteria and simultaneously prevent their adhesion to the catheter surface. This idea derives from the fact that current commercially marketed strategies such as killing bacteria or merely delaying bacterial attachment have been unsuccessful in the long term prevention of CAUTIs.[37] Generally, planktonic bacteria come across a surface submerged in the fluid and within minutes become attached. As long as viable cells remain, they will regrow as soon as they are exposed to nutrient-containing infusion.[38] In this paper, we offer the concept of developing an anti-infective Ag-PTFE coating by combining antibacterial Ag and antiadhesive PTFE via a facile wet chemistry method. By immersing the catheter into the coating bath, both luminal and external surfaces can be coated, ideal for preventing CAUTI as the infection may arise from bacterial invasion from either surface.

Surface characterization studies confirmed the deposition of Ag and PTFE particles onto silicone catheter surfaces, and remarkable changes in surface hydrophilicity and surface energy were observed (Table 1). To examine the anti-infection performance of the coatings, an in vitro adhesion assay was

DOI: 10.1021/acsbiomaterials.9b00071
ACS Biomater. Sci. Eng. 2019, 5, 2804−2814

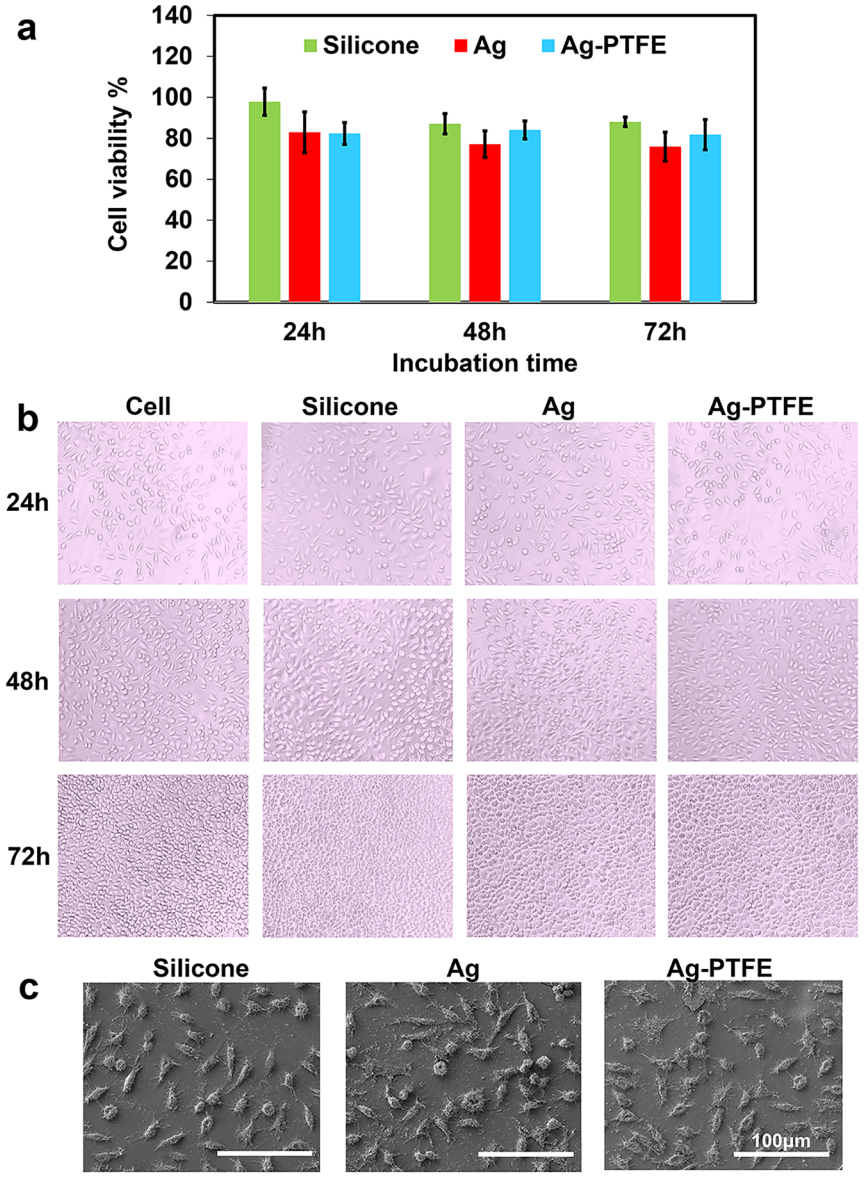


Figure 7. (a) Cell viabilities of L929 mouse fibroblasts after exposing to different catheters for 24, 48 and 72 h; (b) optical images of L929 mouse fibroblasts exposed to different catheters for 24, 48 and 72 h; (c) SEM observations of the fibroblasts after culturing on different catheter surfaces for 72h. Typical images are shown from several examinations (bars represent standard deviation of the mean).

performed with the two most common uropathogens (*E. coli* and *S. aureus*). For either Ag- or Ag-PTFE-coated catheters, the results confirmed that the presence of Ag conferred the catheter surfaces with potent antibacterial properties (Figures 2, 3 and 4b). Comparatively, the Ag-PTFE-coated catheter was shown to further reduce ∼50% of bacterial adhesion when compared to the Ag-coated catheters, and the incorporation of PTFE into the Ag coating did not compromise its antibacterial activity. Interestingly, a difference in the antibacterial efficacy between *E. coli* and *S. aureus* was observed, because over 70% of attached *E. coli* were killed after 24 h of coincubation while this ratio dropped to only about 30% for *S. aureus*. This may be ascribed to the thick peptidoglycan layer of Gram-positive bacteria that prevents the action of the silver ions through the bacterial cell wall.[39] Specifically, the cell walls of Gram-positive bacteria are composed of a rigid and complex network of peptidoglycan along with covalently bound carbohydrates and cell wall associated proteins (40−80 nm), which is drastically thicker than the single layered 7−8 nm thick cell wall of Gram-negative bacteria.[40,41] For this reason, the thicker cell wall of Gram-positive bacteria acts as a barrier protecting the cell from Ag^+ penetration into the cytoplasm.[42] In addition, Kawahara et al.[43] reported that Gram-positive bacteria possess more negatively charged peptidoglycan molecules that can probably bind the Ag^+ that allow less Ag^+ to reach the plasma membrane than Gram-negative species. Sütterlin et al.[44] showed that a minimal bactericidal concentration (MBC) of Ag^+ for Gram-positive bacteria was more than 32 times higher than the MBC values for the Gram-negative bacterial cells.

Theoretically, bacterial adhesion could be explained by the classical Derjaguin−Landau−Verwey−Overbeek (DLVO) theory.[45] According to Baier[46] and Zhao,[47] the optimum surface energy of a surface for which bacterial adhesion force is minimal is approximately 25 mJ/m^2. In this study, the incorporation of low-surface-energy PTFE (∼18 mJ/m^2) into the Ag matrix significantly reduced the total surface energy

DOI: 10.1021/acsbiomaterials.9b00071
ACS Biomater. Sci. Eng. 2019, 5, 2804−2814

(γ^{TOT}) of the coating, which may explain the improved antiadhesive performance of the Ag-PTFE coating. However, it should be noticed that not all the adhered bacteria were killed in this assay, indicating the Ag-PTFE-coated catheter is not meant to treat infections but rather to limit bacteria adhesion and colonization. To further verify the anti-infection effectiveness of the Ag-PTFE-coated catheter, we compared its antibiofilm efficacy with two types of commercialized catheters (BARD PTFE-coated Foley catheters and Mediplus all-silicone Foley catheters). Upon challenge with high-density bacterial suspensions (~2 × 10^8 CFU/mL) in a biofilm-promoting medium (TSB) for 48 h, the Ag-PTFE-coated catheter still demonstrated excellent antibiofilm efficacy in that only separated colonies were found on its surface, while the uncoated commercial silicone catheter was fully covered by biofilm and the PTFE-coated catheter (BARD PTFE) surface also experienced heavy bacterial colonization.

To test the efficacy of our Ag-PTFE coating in a realistic environment, we utilized an in vitro bladder model to mimic the common process of CAUTI. Clinically, the diagnosis of CAUTI is based on finding bacteriuria and the presence of 10^2CFU/mL in urine taken from urinary catheters is considered positive for bacteriuria and would require catheter replacement and prompt antibiotic treatment.[7,48] In comparison with the uncoated silicone catheter, our Ag-PTFE-coated catheter impeded the bacterial migration and extended catheter life from a mean of 6 days to 40 days upon challenge with ~10^2 CFU/mL of *E. coli*. Interestingly, the Ag-PTFE coating also had an effect on resisting bacterial growth as indicated by a significant decrease in biomass accumulation along the catheter surface. Generally, the biofilm mode of growth is a basic survival strategy deployed by bacteria while human urine is a rich medium that supports bacterial growth.[49] Previous in vitro studies have shown that migration does not take place only when the bacterial growth is inhibited.[50] Compared to the uncoated silicone catheter shown to suffer from heavy accumulation of biofilm, the Ag-PTFE coating developed here exhibited both antibacterial and antiadhesive activities that significantly suppressed the spread of bacterial biofilm along the catheter surface. These results further reveal that the Ag-PTFE-coated catheter might satisfy long-term anti-infective needs.

Cytotoxicity results suggest that our Ag-PTFE coating may not be cytotoxic to mammalian cells. To some extent this result is not surprising, considering that both silver and PTFE are FDA-approved biomaterials for urinary catheter coatings.[51,52] However, previous studies have indicated that an overdose of Ag can induce cytotoxicity toward mammal cells due to the fast release and storage of toxic Ag^+ and the adherence of AgNP on cell membrane may further induce cell apoptosis.[53] In our experiments, the release of Ag^+ from the Ag-PTFE-coated catheters proceeded in a two-phase manner that large amounts of Ag^+ were released in the initial 3 days and it became relatively slower and steady during the next 10 days. As evidenced by the cell experiments, the Ag-PTFE-coated catheters do not exhibit deleterious effects on viability of the L929 mouse fibroblast cells during the 3 days of culture, and in fact the Ag-PTFE-coated surfaces result in improved cell attachment and spreading. Although the detailed mechanism still requires further study, improvement in surface hydrophilicity seems to be a common reason for the good cell spreading, which may further aid to combat infection by winning the '"race for the surface against bacteria".[54]

CONCLUSION

In this paper, we have demonstrated a facile and cost-effective approach to produce Ag-PTFE coatings for urinary catheters by incorporating antiadhesive PTFE nanoparticles into an antibacterial Ag matrix. Surface analysis indicated that the PTFE particles were uniformly distributed throughout the Ag-PTFE coating. Compared to the uncoated commercial silicone catheters and the Ag-coated catheters, the Ag-PTFE-coated catheters reduced bacterial adhesion by up to 60.3% and 55.2%, respectively. Compared to the commercial silicone catheters and PTFE-coated catheters, the Ag-PTFE-coated catheters reduced biofilm coverage by up to 97.4% and 96.2%, respectively. The Ag-PTFE-coated catheters also exhibited remarkable antibacterial activity against planktonic bacteria. The data presented in this study showed that the Ag-PTFE coatings possess good stability and prolonged antibacterial activity over a period of 14 days. The Ag-PTFE-coated catheters demonstrated superior antibiofilm efficacy and effectively inhibited bacterial migration and growth along the external catheter surface in a bladder model, extending the lifetime of silicone catheters from a mean of 6 days to over 40 days. Studies with mouse fibroblast cells demonstrated the excellent biocompatibility of the Ag-PTFE-coated catheters. The positive results obtained in this study shows that the Ag-PTFE-coated catheters have the potential to reduce CAUTI.

AUTHOR INFORMATION

Corresponding Author

*E-mail: q.zhao@dundee.ac.uk.

ORCID

Geoffrey Michael Gadd: 0000-0001-6874-870X

Qi Zhao: 0000-0002-4831-1727

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by the Engineering and Physical Sciences Research Council [EPSRC, EP/P00301X/1].

REFERENCES

(1) Nicolle, L. E. Catheter associated urinary tract infections. *Antimicrob. Resist. Infect. Control.* **2014**, *3*, 23.

(2) Saint, S.; Fowler, K. E.; Sermak, K.; Gaies, E.; Harrod, M.; Holland, P.; Bradley, S. F.; Hancock, J. B.; Krein, S. L. Introducing the No Preventable Harms campaign: Creating the safest health care system in the world, starting with catheter-associated urinary tract infection prevention. *Am. J. Infect. Control* **2015**, *43* (3), 254−259.

(3) Yu, K.; Lo, J. C.; Yan, M.; Yang, X.; Brooks, D. E.; Hancock, E. E.; Lange, D.; Kizhakkedathu, J. N. Anti-adhesive antimicrobial peptide coating prevents catheter associated infection in a mouse urinary infection model. *Biomaterials* **2017**, *116*, 69−81.

(4) Feneley, R. C.; Hopley, I. B.; Wells, P. N. Urinary catheters: history, current status, adverse events and research agenda. *J. Med. Eng. Technol.* **2015**, *39* (8), 459−70.

(5) Trautner, B. W.; Darouiche, R. D. Role of biofilm in catheter-associated urinary tract infection. *Am. J. Infect. Control* **2004**, *32* (3), 177−183.

(6) Johnson, L. R. Microcolony and biofilm formation as a survival strategy for bacteria. *J. Theor. Biol.* **2008**, *251* (1), 24−34.

(7) Hooton, T. M.; Bradley, S. F.; Cardenas, D. D.; Colgan, R.; Geerlings, S. E.; Rice, J. C.; Saint, S.; Schaeffer, A. J.; Tambayh, P. A.; Tenke, P.; Nicolle, L. E. Diagnosis, Prevention, and Treatment of Catheter-Associated Urinary Tract Infection in Adults:2009 Interna-

DOI: 10.1021/acsbiomaterials.9b00071
ACS Biomater. Sci. Eng. 2019, 5, 2804−2814

tional Clinical Practice Guidelines from the Infectious Diseases Society of America. *Clin. Infect. Dis.* **2010**, *50* (5), 625−663.
(8) Bridier, A.; Briandet, R.; Thomas, V.; Dubois-Brissonnet, F. Resistance of bacterial biofilms to disinfectants: a review. *Biofouling* **2011**, *27* (9), 1017−1032.
(9) Nguyen, A.; Werneburg, G.; Kim, J.; Rohan, A.; Thanassi, D. MP23−17 The characteristics and progression of bacterial biofilms on urinary catheters. *J. Urol.* **2017**, *197* (4), 300.
(10) Donlan, R. M. Biofilms and device-associated infections. *Emerging Infect. Dis.* **2001**, *7* (2), 277−281.
(11) Lim, K.; Chua, R. R.; Ho, B.; Tambyah, P. A.; Hadinoto, K.; Leong, S. S. Development of a catheter functionalized by a polydopamine peptide coating with antimicrobial and antibiofilm properties. *Acta Biomater.* **2015**, *15*, 127−138.
(12) Chen, R.; Willcox, M. D.; Ho, K. K.; Smyth, D.; Kumar, N. Antimicrobial peptide melimine coating for titanium and its in vivo antibacterial activity in rodent subcutaneous infection models. *Biomaterials* **2016**, *85*, 142−151.
(13) Lawrence, E. L.; Turner, I. G. Materials for urinary catheters: a review of their history and development in the UK. *Med. Eng. Phys.* **2005**, *27* (6), 443−53.
(14) Pickard, R.; Lam, T.; MacLennan, G.; Starr, K.; Kilonzo, M.; McPherson, G.; Gillies, K.; McDonald, A.; Walton, K.; Buckley, B.; Glazener, C.; Boachie, C.; Burr, J.; Norrie, J.; Vale, L.; Grant, A.; N'Dow, J. Antimicrobial catheters for reduction of symptomatic urinary tract infection in adults requiring short-term catheterisation in hospital: a multicentre randomised controlled trial. *Lancet* **2012**, *380* (9857), 1927−1935.
(15) Milo, S.; Thet, N. T.; Liu, D.; Nzakizwanayo, J.; Jones, B. V.; Jenkins, A. T. A. An in-situ infection detection sensor coating for urinary catheters. *Biosens. Bioelectron.* **2016**, *81*, 166−172.
(16) Campoccia, D.; Montanaro, L.; Speziale, P.; Arciola, C. R. Antibiotic-loaded biomaterials and the risks for the spread of antibiotic resistance following their prophylactic and therapeutic clinical use. *Biomaterials* **2010**, *31* (25), 6363−6377.
(17) Trautner, B. W.; Hull, R. A.; Darouiche, R. O. Prevention of catheter-associated urinary tract infection. *Curr. Opin. Infect. Dis.* **2005**, *18* (1), 37−41.
(18) Cook, G.; Costerton, J. W.; Darouiche, R. O. Direct confocal microscopy studies of the bacterial colonization in vitro of a silver-coated heart valve sewing cuff. *Int. J. Antimicrob. Agents* **2000**, *13* (3), 169−173.
(19) Stickler, D. J.; Morgan, S. D. Observations on the development of the crystalline bacterial biofilms that encrust and block Foley catheters. *J. Hosp. Infect.* **2008**, *69* (4), 350−360.
(20) Kazmierska, K. A.; Thompson, R.; Morris, N.; Long, A.; Ciach, T. In Vitro Multicompartmental Bladder Model for Assessing Blockage of Urinary Catheters: Effect of Hydrogel Coating on Dynamics of Proteus mirabilis Growth. *Urology.* **2010**, *76* (2), 515−520.
(21) Zhao, Q.; Liu, Y.; Wang, C. Development and evaluation of electroless Ag-PTFE composite coatings with anti-microbial and anti-corrosion properties. *Appl. Surf. Sci.* **2005**, *252* (5), 1620−1627.
(22) Guo, R.; Yin, G.; Sha, X.; Zhao, Q.; Wei, L.; Wang, H. The significant adhesion enhancement of Ag-polytetrafluoroethylene antibacterial coatings by using of molecular bridge. *Appl. Surf. Sci.* **2015**, *341*, 13−18.
(23) Zhao, Q.; Liu, Y.; Müller-Steinhagen, H.; Liu, G. Graded Ni-P-PTFE coatings and their potential applications. *Surf. Coat. Technol.* **2002**, *155* (2−3), 279−284.
(24) Liu, C.; Zhao, Q. The CQ ratio of surface energy components influences adhesion and removal of fouling bacteria. *Biofouling* **2011**, *27* (3), 275−85.
(25) Lara, H. H.; Núñez, N. V. A.; Turrent, L. C. D.; Padilla, C. R. Bacterialcidal effect of silver nanoparticles against multidrug-resisrant bacteria. *World J. Microbiol. Biotechnol.* **2010**, *26*, 615−621.
(26) Darouiche, R. O.; Safar, H. R.; et al. In Vitro Efficacy of Antimicrobial-Coated Bladder Catheters in Inhibiting Bacterial Migration along Catheter Surface. *J. Infect. Dis.* **1997**, *176* (4), 1109−1112.
(27) Nicolle, L. E.; Bradley, S.; Colgan, R.; Rice, J. C.; Schaeffer, A.; Hooton, T. M. Infectious Diseases Society of America Guidelines for the Diagnosis and Treatment of Asymptomatic Bacteriuria in Adults. *Clin. Infect. Dis.* **2005**, *40* (5), 643−654.
(28) Onat, B.; Bütün, V.; Banerjee, S.; Goktepe, I. Bacterial anti-adhesive and pH-induced antibacterial agent releasing ultra-thin films of zwitterionic copolymer micelles. *Acta Biomater.* **2016**, *40*, 293−309.
(29) Poskus, L. T.; Lima, R.; Lima, I.; Guimarães, J.; Silva, E.; Granjeiro, J. Cytotoxicity of current adhesive systems: in vitro testing on cell culture of L929 and balb/c 3T3 fibroblasts. *Rev. odonto.ciênc.* **2009**, *24*, 129−134.
(30) Mei, S.; Wang, H.; Wang, W.; Tong, L.; Pan, H.; Ruan, C.; Ma, Q.; Liu, M.; Yang, H.; Zhang, L.; Cheng, Y.; Zhang, Y.; Zhao, L.; Chu, P. K. Antibacterial effects and biocompatibility of titanium surfaces with graded silver incorporation in titania nanotubes. *Biomaterials* **2014**, *35* (14), 4255−4265.
(31) Liu, C.; Geng, L.; Yu, Y.; Zhang, Y.; Zhao, B.; Zhao, Q. Mechanisms of the enhanced antibacterial effect of Ag-TiO_2 coatings. *Biofouling* **2018**, *34* (2), 190−199.
(32) Dhanalekshmi, K. I.; Meena, K. S. Comparison of antibacterial activities of Ag@TiO_2 and Ag@SiO_2 core-shell nanoparticles. *Spectrochim. Acta, Part A* **2014**, *128*, 887−890.
(33) Agnihotri, S.; Mukherji, S.; Mukherji, S. Immobilized silver nanoparticles enhance contact killing and show highest efficacy: elucidation of the mechanism of bactericidal action of silver. *Nanoscale* **2013**, *5* (16), 7328−7340.
(34) Shah, J. Catheterization. *Ann. R. Coll. Surg. Engl.* **2012**, *94*, 5−7.
(35) Ozdemir, K. G.; Yilmaz, H.; Yilmaz, S. In vitro evaluation of cytotoxicity of soft lining materials on L929 cells by MTT assay. *J. Biomed. Mater. Res., Part B* **2009**, *90B* (1), 82−86.
(36) Nie, C.; Yang, Y.; Cheng, C.; Ma, L.; Deng, J.; Wang, L.; Zhao, C. Bioinspired and biocompatible carbon nanotube-Ag nanohybrid coatings for robust antibacterial applications. *Acta Biomater.* **2017**, *51*, 479−494.
(37) Levering, V.; Cao, C.; Shivapooja, P.; Levinson, H.; Zhao, X.; López, G. P. Urinary catheter capable of repeated on-demand removal of infectious biofilms via active deformation. *Biomaterials* **2016**, *77*, 77−86.
(38) Roe, D.; Karandikar, B.; Bonn, S. N.; Gibbins, B.; Roullet, J. B. Antimicrobial surface functionalization of plastic catheters by silver nanoparticles. *J. Antimicrob. Chemother.* **2008**, *61* (4), 869−876.
(39) Jung, W. K.; et al. Antibacterial Activity and Mechanism of Action of the Silver Ion in Staphylococcus aureus and Escherichia coli. *Appl. Environ. Microbiol.* **2008**, *74*, 2171−2178.
(40) Malanovic, N.; Lohner, K. Gram-positive bacterial cell envelopes: The impact on the activity of antimicrobial peptides. *Biochim. Biophys. Acta, Biomembr.* **2016**, *1858* (5), 936−46.
(41) Mitchell, G. J.; Wiesenfeld, K.; Nelson, D. C.; Weitz, J. S. Critical cell wall hole size for lysis in Gram-positive bacteria. *J. R. Soc., Interface* **2013**, *10* (80), 20120892.
(42) López-Heras, M.; Theodorou, I. G.; Leo, B. F.; Ryan, M. P.; Porter, A. E. Towards understanding the antibacterial activity of Ag nanoparticles: electron microscopy in the analysis of the materials-biology interface in the lung. *Environ. Sci.: Nano* **2015**, *2*, 312−326.
(43) Kawahara, K.; Tsuruda, K.; Morishita, M.; Uchida, M. Antibacterial effect of silver-zeolite on oral bacteria under anaerobic conditions. *Dent. Mater.* **2000**, *16* (6), 452−455.
(44) Sütterlin, S.; Tano, E.; Bergsten, A.; Tallberg, A. B.; Melhus, A. Effects of silver-based wound dressings on the bacterial flora in chronic leg ulcers and its susceptibility in vitro to silver. *Acta Derm. Venereol.* **2012**, *92* (1), 34−9.
(45) Hermansson, M. The DLVO theory in microbial adhesion. *Colloids Surf., B* **1999**, *14*, 105−119.
(46) Baier, R. E. Surface behaviour of biomaterials: The theta surface for biocompatibility. *J. Mater. Sci.: Mater. Med.* **2006**, *17*, 1057−1062.
(47) Zhao, Q.; Müller-Steinhagen, H. *Intermolecular and Adhesion Forces of Deposits on Modified Heat Transfer Surfaces;* United

DOI: 10.1021/acsbiomaterials.9b00071
ACS Biomater. Sci. Eng. 2019, 5, 2804−2814

Engineering Foundation Conference, Fundamental Approaches and Technical Solutions: Davos, Switzerland, 2001.
(48) de Cueto, M.; Aliaga, L.; Alós, J. I.; Canut, A.; Los-Arcos, I.; Martínez, J. A.; Mensa, J.; Pintado, V.; Rodriguez-Pardo, D.; Yuste, J. R.; Pigrau, C. Executive summary of the diagnosis and treatment of urinary tract infection: Guidelines of the Spanish Society of Clinical Microbiology and Infectious Diseases (SEIMC). *Enferm. Infecc. Microbiol. Clin.* **2017**, *35* (5), 314−320.
(49) Stickler, D. J. Bacterial biofilms in patients with indwelling urinary catheters. *Nat. Clin. Pract. Urol.* **2008**, *5* (11), 598−608.
(50) Harkes, G.; Dankert, J.; Feijen, J. Bacterial Migration along Solid Surfaces. *Appl. Environ. Microbiol.* **1992**, *58*, 1500−1505.
(51) Singha, P.; Locklin, J.; Handa, H. A review of the recent advances in antimicrobial coatings for urinary catheters. *Acta Biomater.* **2017**, *50*, 20−40.
(52) Marjanović-Balaban, Z.; Jelić, D. Polymeric materials in clinical practice. In *Biomaterials in Clinical Practice: Advances in Clinical Research and Medical Devices*; Zivic, F., Affatato, S., Trajanovic, M., Schnabelrauch, M., Grujovic, N., Choy, K. L. Springer International Publishing: Cham, Switzerland, 2018; pp 106−107.
(53) Kim, Y. J.; Yang, S. I.; Ryu, J. Cytotoxicity and genotoxicity of nano-silver in mammalian cell lines. *Mol. Cell. Toxicol.* **2010**, *6*, 119−125.
(54) Gao, A.; Hang, R.; Huang, X.; Zhao, L.; Zhang, X.; Wang, L.; Tang, B.; Ma, S.; Chu, P. K. The effects of titania nanotubes with embedded silver oxide nanoparticles on bacteria and osteoblasts. *Biomaterials* **2014**, *35* (13), 4223−4235.

DOI: 10.1021/acsbiomaterials.9b00071
ACS Biomater. Sci. Eng. 2019, 5, 2804−2814